Cheryl

PATENT

#8

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In Re: U.S. Patent 4,427,663

Issued: January 24, 1984

To: Helmut Mrozik

For: 4"-KETO-AND 4"-AMINO-4"-DEOXY SUBSTITUTED AMINO DERIVATIVES THEREOF

Commissioner of Patents and Trademarks
Box Patent Extension
Washington, D. C. 20231

Re: Deposit Account 13-2755
MERCK & CO., Inc.
U.S. Patent 4,427,663

Sir:

Transmitted herewith is the application for extension of patent term under 35 U.S.C. 156 with regard to U.S. Patent 4,427,663

Please charge our Deposit Account No. 13-2755 in the amount of $1,090.00. The Commissioner is hereby authorized to charge any additional fees, which may be required, or credit any overpayment to Account No. 13-2755. Duplicate copies of this sheet are enclosed.

Respectfully submitted,

By: _____
Mollie M. Yang
Reg. No. 32,718
Attorney for Applicant(s)

MERCK & CO., INC.
P.O. Box 2000
Rahway, New Jersey 07065-0907
(908) 594-6343

Date: 10 June 1997

IN TRIPLICATE

PATENT

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In Re: U.S. Patent 4,427,663

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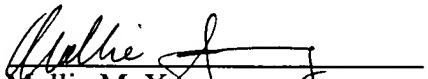
By: 
Mollie M. Yang
Reg. No. 32,718
Attorney for Applicant(s)

MERCK & CO., INC.
P.O. Box 2000
Rahway, New Jersey 07065-0907
(908) 594-6343

Date: 16 June 1997

IN TRIPLICATE

PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In Re: U.S. Patent 4,427,663

Issued: January 24, 1984

To: Helmut Mrozik

For: 4"-KETO-AND 4"-AMINO-4"-DEOXY SUBSTITUTED AMINO DERIVATIVES THEREOF

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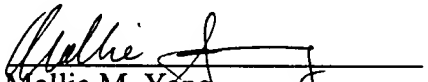
Respectfully submitted,

By: 
Mollie M. Yang
Reg. No. 32,718
Attorney for Applicant(s)

MERCK & CO., INC.
P.O. Box 2000
Rahway, New Jersey 07065-0907
(908) 594-6343

Date: 10 June 1997

IN TRIPLICATE

PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In Re:	U.S. Patent No. 4,427,663
Issued.:	January 24, 1984
To:	Helmut Mrozik
For:	4"-KETO-AND 4"-AMINO-4"-DEOXY AVERMECTIN COMPOUNDS AND SUBSTITUTED AMINO DERIVATIVES THEREOF

Assistant Commissioner for Patents
Washington, D.C. 20231
ATTN: Box Patent Extension

APPLICATION FOR EXTENSION OF PATENT TERM UNDER 35 U.S.C. 156

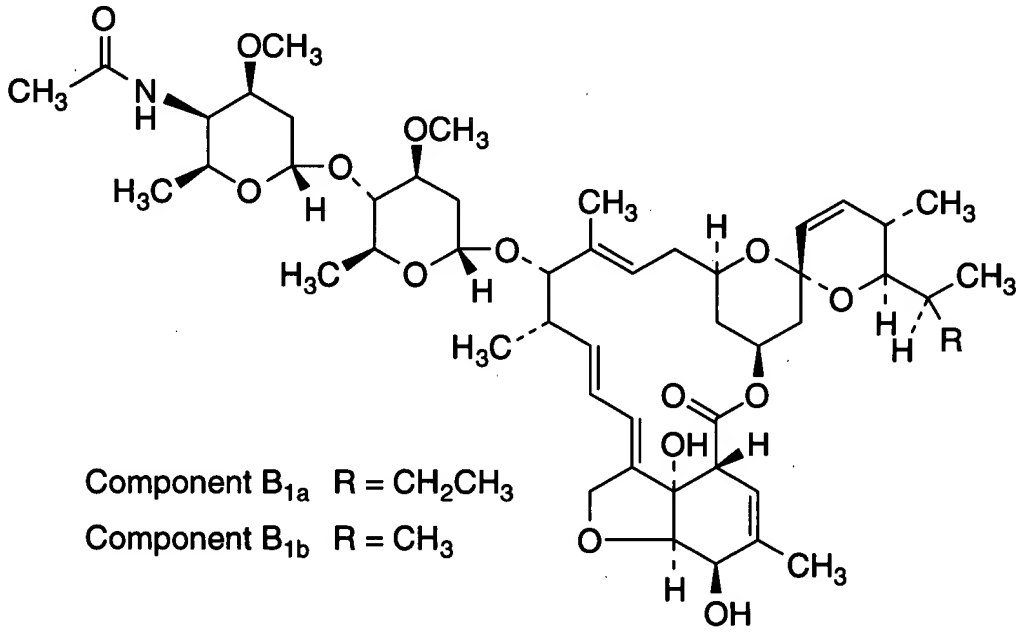
Sir:

Your Applicant, Merck & Co., Inc., a corporation organized and existing under the laws of the state of New Jersey, represents that it is the assignee of the entire interest in and to Letters Patent of the United States No. 4,427,663 granted to Helmut Mrozik on 24 January 1984 for 4"-KETO-AND 4"-AMINO-4"DEOXY AVERMECTIN COMPOUNDS AND SUBSTITUTED AMINO DERIVATIVES THEREOF" by virtue of an assignment in favor of Merck & Co., Inc. recorded November 2, 1983, Reel No. 418, Frame No. 726. Your Applicant, acting through its duly authorized attorney, hereby submits this application for extension of patent term under 35 U.S.C. 156 by providing the following information required by the rules promulgated by the U.S. Patent and Trademark Office (37 C.F.R. 1.740). For the convenience of the Patent and Trademark Office, the information contained in this application will be presented in a format which will follow the requirements of Section 1.740 of Title 37 of the Code of Federal Regulations.

(1) IVOMEC® EPRINEX™ Pour-On for Beef and Dairy Cattle which contains as the active ingredient, eprinomectin, consists of at least 90% by weight of the Bla component and no more than 10% of the Blb component. The chemical name for each component is as follows:

B1a = 4"-epiacetylamino-4"-deoxyavermectin Bla; or (4"R)-4"-(acetylamino)-5-O-demethyl-4"-deoxyavermectin A1a.

Blb = 4"-epiacetylamino-4"-deoxyavermectin Blb; or (4"R)-4"-(acetylamino)-5-O-demethyl-25-de(1-methylpropyl)-4"-deoxy-25-(1-methylethyl)avermectin A1a.



(2) The approved product was subject to regulatory review under the Federal Food, Drug and Cosmetic Act Section 512 (21 U.S.C. 360b).

(3) The approved product, IVOMEC® EPRINEX™ Pour-On for Beef and Dairy Cattle, received permission for commercial marketing or use under section 512 of the Federal Food, Drug and Cosmetic Act (21 U.S.C. 360b) on 16 April 1997.

(4) The only active ingredient in IVOMEC® EPRINEX™ Pour-On for Beef and Dairy Cattle is eprinomectin which has not been approved for commercial marketing or use under Section 512 of the Federal Food, Drug and Cosmetic Act prior to the approval of NADA 141-079 by the Food and Drug Administration on 16 April 1997.

(5) This application for extension of patent term under 35 U.S.C. 156 is being submitted within the permitted 60 day period pursuant to 37 C.F.R. 1.720(f), said period which will expire on 16 June 1997.

(6) The complete identification of the patent for which extension is being sought is as follows:

Inventor: Helmut Mrozik

Patent Number: U.S. Patent 4,427,663

Issue Date: January 24, 1984

Expiration Date: March 16, 2002, as determined by 35 U.S.C. 154(c) enacted pursuant to the General Agreement of Tariffs and Trade (GATT), [Pub. L. No. 103-465 (H.R.5110), signed December 8, 1994, effective January 1, 1995]. Note, the original expiration date of the patent, prior to 35 U.S.C. 154(c) implementation, would be January 24, 2001.

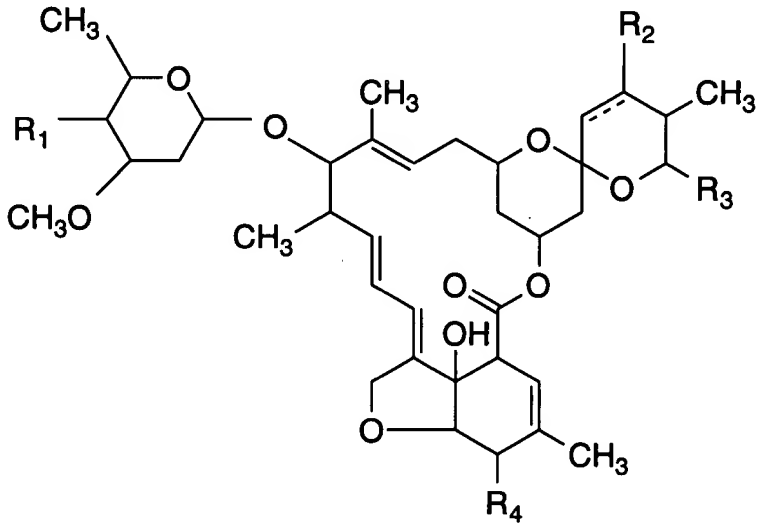
(7) See "Attachment A" for a complete copy of the patent identified in paragraph (6) hereof.

(8) No Terminal Disclaimer has been issued with regard to U.S. Patent 4,427,663. No Certificate of Correction or Re-examination Certificate has been issued with regard to U.S. Patent 4,427,663. The receipt for the third maintenance fee payment made in 1995 for US Patent 4,427,663 is attached hereto as "Attachment B".

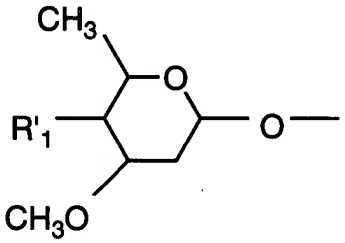
(9) U.S. Patent 4,427,663 claims the approved product. Specifically, the active ingredient, eprinomectin, is claimed in Claims 1, 2 and 6; the method of treatment utilizing the active ingredient, eprinomectin, is claimed in Claim 13; and the pharmaceutical composition containing the active ingredient, eprinomectin, is claimed in Claim 14.

Claim 1 reads as follows:

1. A compound having the formula:



wherein R_1 is =O, -NR5R6 or



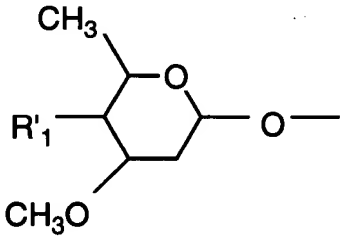
wherein

R'_1 is =O or -NR5R6;

R5 and R6 are independently hydrogen, loweralkyl, loweralkanoyl, substituted benzenesulfonyl wherein the substituent is halogen; or loweralkyl sulfonyl;

R_2 is hydrogen or hydroxy;
R_3 is sec-butyl or iso-propyl;
R_4 is hydroxy or methoxy;
and the broken line indicates a single or a double bond at the 22,23-position provided that R_2 can only be hydroxy when the broken line indicates a single bond.

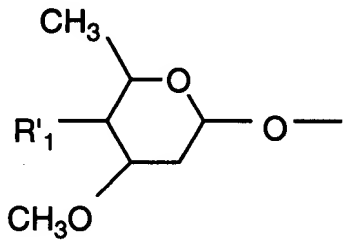
The approved product contains eprinomctin which is a mixture of the B1a and B1b components which is a compound of Claim 1 wherein R_3 is sec-butyl for the B1a component and R_3 is isopropyl for the B1b component; R_2 is hydrogen; R_1 is



R'_1 is-NR_5R_6; R_5 is hydrogen and R_6 is loweralkanoyl; and the broken line indicates a double bond at the 22,23-position.

Claim 2 reads as follows:

2. The compound of Claim 1 wherein R_1 is:



and R'_1 is =O, or -NR_5R_6 wherein R_5 and R_6 are independently hydrogen, methyl or acetyl.

The approved product contains eprinomectin which is a mixture of the B1a and B1b components which is a compound of Claim 2 wherein R'_1 is -NR_5R_6; and R_5 is hydrogen and R_6 is acetyl.

Claim 6 reads as follows:

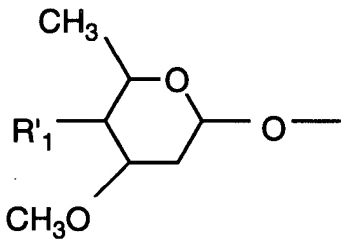
6. The compound of Claim 2 wherein R'_1 is -NR_5R_6 and R_5 and R_6 are indepenently hydrogen, methyl or acetyl.

The approved product contains eprinomectin which is a mixture of the B1a and B1b components which is a compound of Claim 6 wherein R_5 is hydrogen, and R_6 is acetyl.

Claim 13 reads as follows:

13. A method for the treatment of helminthiasis which comprises administering to an animal infected with helminths an effective amount of a compound of claim 1.

The approved product is used for the treatment of helminthiasis by treating an animal infected with helminths with eprinomectin which is a mixture of the B1a and B1b components which is a compound of Claim 1 wherein R_3 is sec-butyl for the B1a component and R_3 is isopropyl for the B1b component; R_2 is hydrogen; R_1 is



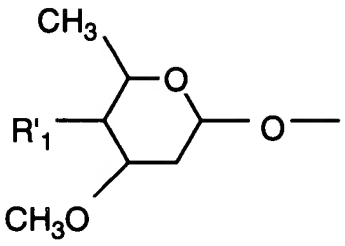
R'_1 is-NR_5R_6; R_5 is hydrogen and R_6 is loweralkanoyl; and the broken line indicates a double bond at the 22,23-position.

Claim 14 reads as follows:

14. A composition useful for treating animals infected with helminths which comprises an inert carrier and an effective amount of a compound of claim 1.

The approved product is a composition containing eprinomectin which is a mixture of the B1a and B1b components which is a compound of Claim 1 wherein R_3 is

sec-butyl for the B1a component and R_3 is isopropyl for the B1b component; R_2 is hydrogen; R_1 is



R'_1 is-NR_5R_6; R_5 is hydrogen and R_6 is loweralkanoyl; and the broken line indicates a double bond at the 22,23-position .

(10) The relevant dates and information pursuant to 35 U.S.C. 156(g) to enable the Secretary of Health and Human Services to determine the applicable regulatory review period are as follows:

(i) A multigeneration study in rats of eprinomectin was initiated on June 22, 1990 and completed on June 20, 1991. This study is a major health or environmental effects test on eprinomection in that (a) it relates to the evaluation of eprinomectin's health effects; (b) it produces data necessary for marketing approval of eprinomectin; and (c) it was conducted over a period of no less than 6 months duration, excluding time required to analyze or evaluate test results. Substantiation of the starting and completion dates of the multigeneration study is included herein as "Attachment C".

An Investigational New Animal Drug Application was submitted on 25 February 1991; and Investigational New Animal Drug (INAD) number 8058 was assigned on 28 February 1991.

For the purpose of determining the duration of patent term restoration for IVOMEC® EPRINEX™ Pour-On for Beef and Dairy Cattle, the date a major health or environmental effects test was initiated, i.e. June 22, 1990 is being used to determine the beginning of the testing phase of the regulatory review period.

(ii) New Animal Drug Application (NADA 141-079) for IVOMEC® EPRINEX™ Pour-On for Beef and Dairy Cattle (eprinomectin) was submitted on 27 March 1997 and

(iii) New Animal Drug Application (NADA 141-079) for IVOMEC® EPRINEX™ Pour-On for Beef and Dairy Cattle (eprinomectin) was approved on 16 April 1997.

(11) As a brief description of the activities undertaken by Applicant, Merck & Co., Inc., during the applicable regulatory review period, attached hereto as "Attachment D" is a chronology of the major activities undertaken by Applicant in seeking the approval of IVOMEC® EPRINEX™ Pour-On for Beef and Dairy Cattle and the major communications between the Applicant and the FDA from 25 February 1991 to 16 April 1997.

(12)(A) Applicant is of the opinion that U.S. Patent 4,427,663 is eligible for extension under 35 U.S.C. 156 because it satisfies all of the requirements for such extension as follows:

(a) 35 U.S.C. 156(a)
U.S. Patent 4,427,663 claims a product and a method of using a product.

(b) 35 U.S.C. 156(a)(1)
The term of the U.S. Patent 4,427,663 has not expired before submission of this application.

(c) 35 U.S.C. 156(a)(2)
The term of U.S. Patent 4,427,663 has never been extended under this provision of the law.

(d) 35 U.S.C. 156(a)(3)
The application for extension is submitted by the owner of record in accordance with the requirement of 35 U.S.C. 156(d) and rules of the U.S. Patent and Trademark Office.

(e) 35 U.S.C. 156(a)(4)
The product, IVOMEC® EPRINEX™ Pour-On for Beef and Dairy Cattle, has been subjected to a regulatory review period before its commercial marketing or use.

(f) 35 U.S.C. 156(a)(5)(A)
The commercial marketing or use of the product, IVOMEC® EPRINEX™ Pour-On for Beef and Dairy Cattle, after the regulatory review period is the first permitted commercial marketing or use of the product under the provision of the Federal Food, Drug and Cosmetic Act (21 U.S.C. 360b) under which such regulatory review period occurred.

(g) 35 U.S.C 156(c)(4)
No other patent has been extended for the same regulatory review period for the product, IVOMEC® EPRINEX™ Pour-On for Beef and Dairy Cattle.

(B) The length of extension of the patent term of U.S. Patent 4,427,663 claimed by Applicant is 1255 days or 3.4 years. The length of the extension was determined pursuant to 37 C.F.R. 1.778 as follows:

(a) The regulatory review period under 35 U.S.C. 156(g)(4)(B) began on 22 June 1990 and ended on 16 April 1997 which is a total of 2490 days or 6.82 years which is the sum of (i) and (ii) below:

(i) The period of review under 35 U.S.C. 156(g)(4)(B)(i), the "Testing Period", began on 22 June 1990 and ended on 27 March 1997, which is 2470 days or 6.77 years; and

(ii) The period of review under 35 U.S.C. 156(g)(4)(B)(ii), the "Application Period", began on 27 March 1997 and ended on 16 April 1997 which is 20 days or 0.05 years;

(b) The regulatory review period upon which the period of extension is calculated is the entire regulatory review period as determined in sub-paragraph 12(B)(a)above (2490 days) less:

(i) The number of days in the regulatory review period which were on or before the date on which the patent issued (January 24, 1984) which is 0 days, and

(ii) The number of days during which applicant did not act with due diligence which is 0 days, and

(iii) One-half of 2470 days which is 1235 days;

(iv) The regulatory review period is calculated by subtracting the number of days determined in sub-paragraph 12(B)(b)(iii) from the entire regulatory review period as determined in sub-paragraph 12(B)(a) (which is 2490 days -1235 days) which equals 1255 days.

(c) The number of days as determined in sub-paragraph 12(B)(b)(iv) (1255 days) when added to the term of the patent (March 16, 2002, as determined by 35 U.S.C. 154(c)) would result in the date, 22 August 2005;

(d) Fourteen (14) years when added to the date of NADA approval (16 April 1997) would result in the date, 16 April 2011.

(e) The earlier date as determined in sub-paragraphs 12(B)(c) and 12(B)(d) is 22 August 2005;

(f) Since the original patent was issued before 24 September 1984, but a request for an exemption was not submitted before 24 September, 1984 and the commercial marketing or use of the product was not approved before 24 September, 1984, five (5) years when added to the expiration date of the patent (March 16, 2002, as determined by 35 U.S.C. 154(c)) would result in the date, March 16 2007

(g) The earlier date as determined in sub-paragraph (12)(B)(e) and (12)(B)(f) is 22 August 2005.

(13) Applicant acknowledges a duty to disclose to the Commissioner of Patents and Trademarks and the Secretary of Health and Human Services any information which is material to the determination of entitlement to the extension sought.

(14) The prescribed fee for receiving and acting upon this application is to be charged to Deposit Account of Applicant is authorized in the attached letter, which is submitted in duplicate.

(15) Correspondence related to this application for extension of the patent term of U.S. Patent 4,427,663 should be addressed to Mollie M. Yang, Reg. No. 32,718 Merck & Co., Inc. P.O. Box 2000, Rahway, New Jersey 07065-0907. Telephone (908) 594-6343.

(16) The instant application for extension of the patent term of U.S. Patent 4,427,663 is being submitted as one original and triplicate copies thereof.

(17) The requisite declaration pursuant to 37 C.F.R. 1.740(b) is attached.

Respectfully submitted,

By _____
Mollie M. Yang
Reg. No. 32,718
Attorney for Applicants
Merck & Co., Inc.
P.O. Box 2000
Rahway, NJ 07065-0907
(908) 594-6343

Date: 10 June 1997

CERTIFICATION

The undersigned hereby certifies that this application for extension of patent term under 35 U.S.C. 156 including its attachments and supporting papers is being submitted as one original and triplicate copies thereof.

_____
Mollie M. Yang

Date: 10 June 1997

PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In Re: U.S. Patent 4,427,663

Issued: January 24, 1984

To: Helmut Mrozik

For: 4"-KETO-AND 4"-AMINO-4"-DEOXY SUBSTITUTED AMINO DERIVATIVES THEREOF

Commissioner of Patents and Trademarks
Box Patent Extension
Washington, D. C. 20231

DECLARATION

Sir:

The undersigned Attorney for Merck & Co., Inc. which is the Applicant for Extension of Patent Term under 35 U.S.C. 156 with regard to U.S. Patent No. 4,427,663 hereby declares as follows:

(1) THAT he is a patent attorney authorized to practice before the Patent and Trademark Office and has general authority from the owner to act on behalf of the owner in patent matters;

(2) THAT he has reviewed and understands the contents of the application being submitted pursuant to 35 U.S.C. 156 and 37 C.F.R. 1.740;

(3) THAT he believes the patent is subject to extension pursuant to 35 U.S.C. 156 and 37 C.F.R. 1.710.

(4) THAT he believes an extension of the length claimed is fully justified under 35 U.S.C. 156.

(5) THAT he believes the patent for which the extension is being sought meets the conditions for extension of the term of a patent as set forth in 35 U.S.C. 156 and 37 C.F.R. 1.720.

The undersigned hereby declares further that all statements made herein of his own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so

made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any extension of patent term issuing thereon.

Further declarant sayeth not.

Signed this 10th day of June 1997.

David L. Rose

David L. Rose

ATTACHMENT A

United States Patent [19]

Mrozik

[11] **4,427,663**

[45] **Jan. 24, 1984**

[54] **4''-KETO-AND 4''-AMINO-4''-DEOXY AVERMECTIN COMPOUNDS AND SUBSTITUTED AMINO DERIVATIVES THEREOF**

[75] Inventor: **Helmut H. Mrozik**, Matawan, N.J.

[73] Assignee: **Merck & Co., Inc.**, Rahway, N.J.

[21] Appl. No.: **358,736**

[22] Filed: **Mar. 16, 1982**

[51] **Int. Cl.**[3] **A61K 31/71**; C07H 15/22

[52] **U.S. Cl.** **424/180**; 536/7.1; 424/181

[58] **Field of Search** 424/180; 536/7.1

[56] **References Cited**

U.S. PATENT DOCUMENTS

4,044,123	8/1977	Daniels et al.	536/13.6
4,085,119	4/1978	Myers .	
4,090,017	5/1978	Sciavolino .	
4,098,993	7/1978	Bright .	
4,107,435	8/1978	Ross	536/13.6
4,133,950	1/1979	Myers .	
4,199,569	4/1980	Chabala et al. .	
4,206,205	6/1980	Mrozik et al. .	
4,310,519	1/1982	Albers-Schonberg et al. .	

OTHER PUBLICATIONS

Chemical Abstracts 91: 175223f to Bright (II).
Chemical Abstracts 90: 23614a to Sciavolino (II).
Chemical Abstracts 90: 23615b to Sciavolino (III).

Primary Examiner—Johnnie R. Brown
Attorney, Agent, or Firm—David L. Rose; Mario A. Monsoo

[57] **ABSTRACT**

There are disclosed novel avermectin compounds wherein the 4'' hydroxy group is oxidized to a keto group or replaced with an amino or substituted amino group. The keto compounds are prepared by oxidation with reagents such as oxalyl chloride in dimethylsulfoxide. The amino compounds are prepared from the ketone using a reducing agent and an aminating agent. Substituted amino compounds are prepared from the thus produced unsubstituted amino compounds. The keto compounds and amino compounds have utility as anti-parasitic agents and compositions for that use are also disclosed. The compounds are also highly potent insecticides against agricultural pests. In addition the amino compounds have anti-bacterial activity which is not found in any of the precursors.

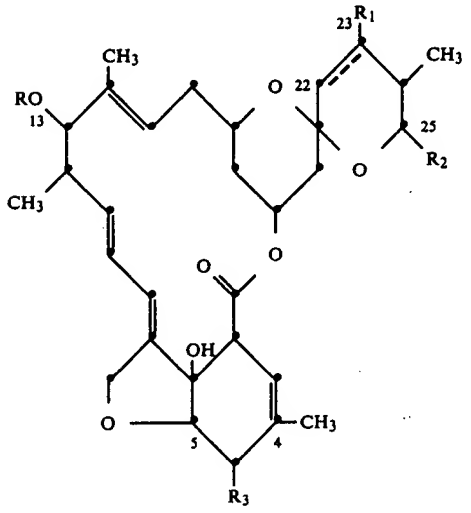
14 Claims, No Drawings

4''-KETO-AND 4''-AMINO-4''-DEOXY AVERMECTIN COMPOUNDS AND SUBSTITUTED AMINO DERIVATIVES THEREOF

BACKGROUND OF THE INVENTION

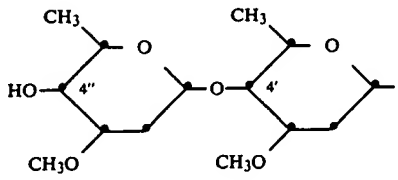
The term avermectin (previously referred to as C-076) is used to describe a series of compounds isolated from the fermentation broth of an avermectin producing strain of *Streptomyces avermitilis* and derivatives thereof. The morphological characteristics of the culture are completely described in U.S. Pat. No. 4,310,519. The avermectin compounds are a series of macrolides, each of which is substituted thereon at the 13-position with a 4-(α-L-oleandrosyl)-α-L-oleandrose group. The avermectin compounds and the instant derivatives thereof have a very high degree of anthelmintic and anti-parasitic activity.

The avermectin series of compounds isolated from the fermentation broth have the following structure:



I

wherein R is the 4'-(α-1-oleandrosyl)-α-1-oleandrose group of the structure:



II

and wherein the broken line indicates a single or a double bond; R_1 is hydroxy and is present only when said broken line indicates a single bond;

R_2 is iso-propyl or sec-butyl; and

R_3 is methoxy or hydroxy.

There are eight different avermectin natural product compounds and they are given the designations A1a, A1b, A2a, A2b, B1a, B1b, and B2a based upon the structure of the individual compounds.

In the foregoing structural formula, the individual avermectin compounds are as set forth below. (The R group is 4'(α-L-oleandrosyl)-α-L-oleandrose):

A1a	Double Bond	sec-butyl	$-OCH_3$
A1b	Double Bond	iso-propyl	$-OCH_3$
A2a	—OH	sec-butyl	$-OCH_3$
A2B	—OH	iso-propyl	$-OCH_3$
B1a	Double Bond	sec-butyl	—OH
B1b	Double Bond	iso-propyl	—OH
B2a	—OH	sec-butyl	—OH
B2b	—OH	iso-propyl	—OH

The avermectin compounds are generally isolated as mixtures of a and b components. Such compounds differ only in the nature of the R_2 substituent and the minor structural differences have been found to have very little effect on the isolation procedures, chemical reactivity and biological activity of such compounds.

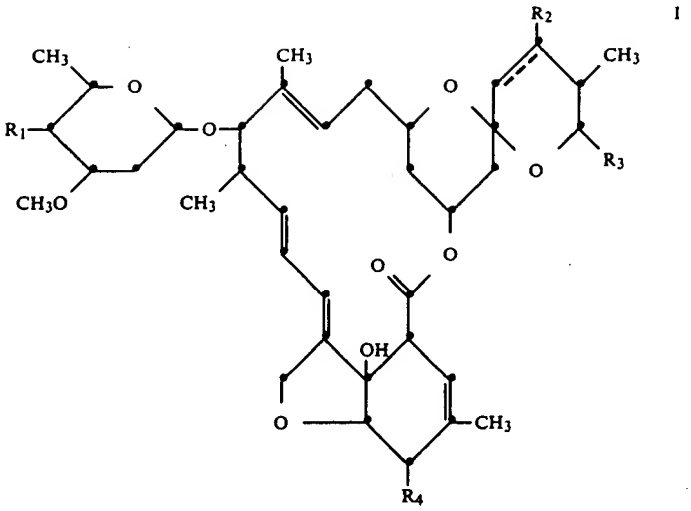
The end hydroxy group of the disaccharide substituent at the 13-position is situated at what is referred to as the 4''-position. The reactions and substitutions at the 4''-position is the subject matter of this invention.

SUMMARY OF THE INVENTION

The instant invention is concerned with certain derivatives of avermectin compounds wherein the 4''-hydroxy group is oxidized to ketone and replaced by an amino or substituted amino group. Thus it is an object of the instant invention to describe such 4''-substituted avermectin compounds. A further object is to describe processes for the preparation of such compounds. A still further object is to describe the uses of such compounds as anti-parasitic agents and anti-bacterial agents. Still further objects will become apparent from a reading of the following description.

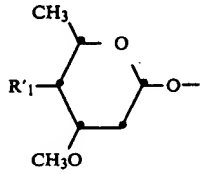
DESCRIPTION OF THE INVENTION

The compounds of the instant invention have the following structural formula.



I

wherein R_1 is =O, $-NR_5R_6$ or



wherein

R'_1 is =O, or $-NR_5R_6$;

R_5 and R_6 are independently hydrogen, loweralkyl, loweralkanoyl, substituted benzenesulfonyl wherein the substitutent is halogen or loweralkyl sulfonyl;

R_2 is hydrogen or hydroxy,

R_2 is iso-propyl or sec-butyl,

R_4 is hydroxy or methoxy,

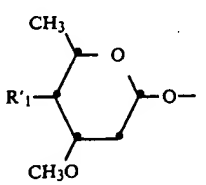
and the broken line indicates a single or a double bond at the 22,23-position, provided that R_2 is hydroxy only when the broken line indicates a single bond.

The term "loweralkyl" when used in the instant application is intended to represent those alkyl groups either straight or branched chain which have from 1–5 carbon atoms. Examples of such alkyl groups are methyl, ethyl, propyl, iso-propyl, butyl, sec-butyl, pentyl, and the like.

The term "loweralkanoyl" is intended to include those alkanoyl groups containing from one to five carbon atoms in either a straight or branched chain. Examples of such alkanoyl groups are formyl, acetyl, propenyl, butyryl, valeryl, and the like.

The term "halogen" is intended to include those halogen atoms fluorine, chlorine, bromine and iodine.

One aspect of the preferred compounds of this invention is realized in the above structural formula when R_1 is

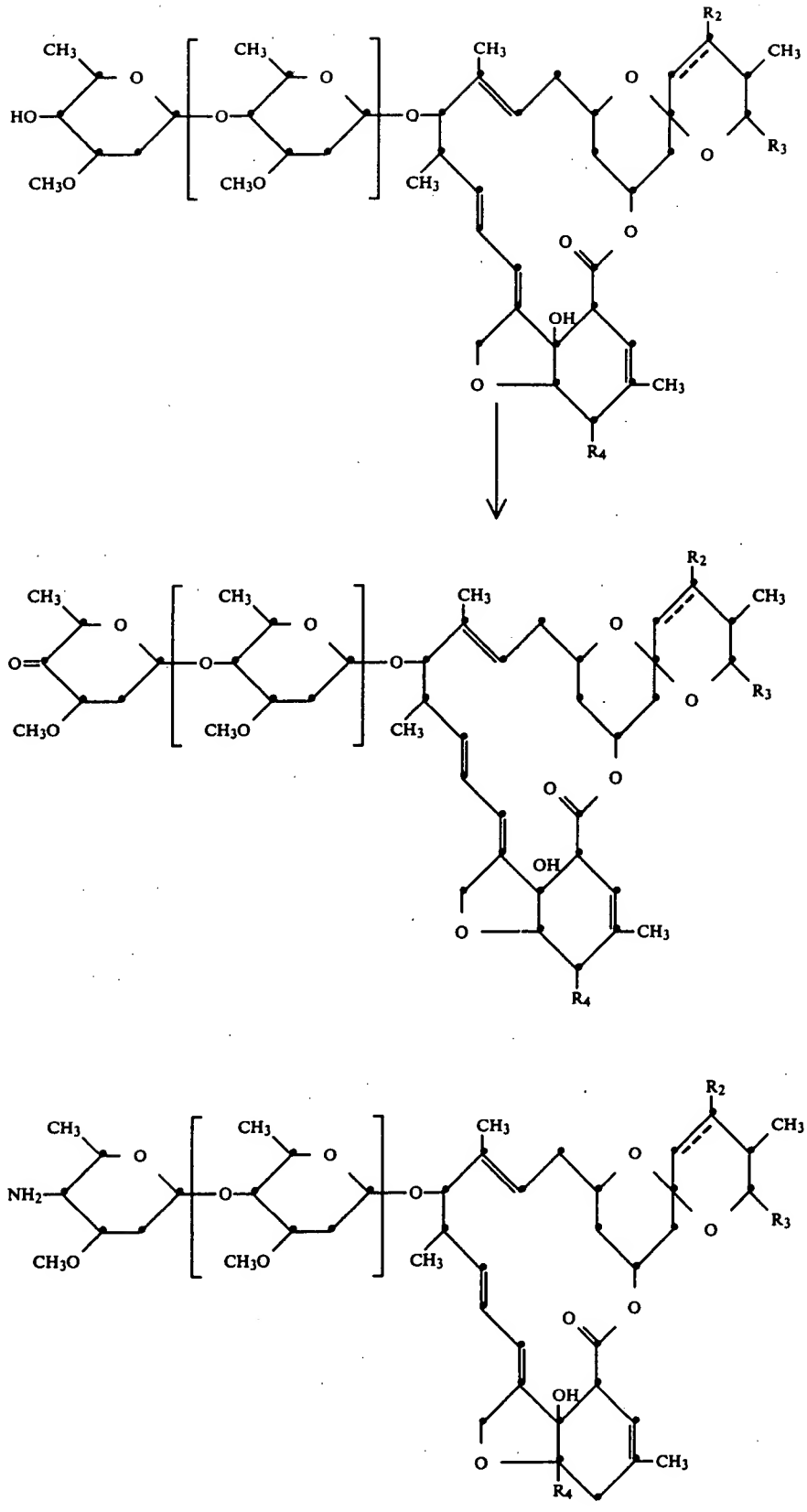


and R'_1 is =O or NR_5R_6 and R_5 and R_6 are independently hydrogen, methyl or acetyl. Further, examples of preferred compounds of the instant invention are:

4"-keto avermectin B1a;
4"-keto avermectin B1b;
4"-keto-22,23-dihydro avermectin B1a;
4"-keto-22,23-dihydro avermectin B1b;
4"-deoxy-4"-amino avermectin B1a;
4"-deoxy-4"-amino avermectin B2b;
4"-deoxy-4"-amino-22,23-dihydro avermectin B1a;
4"-deoxy-4"-amino-22,23-dihydro avermectin B1b;
4"-deoxy-4"-acetylamino avermectin B1a;
4"-deoxy-4"-acetylamino avermectin B1b;
4"-deoxy-4"-acetylamino-22,23-dihydro avermectin B1a;
4"-deoxy-4"-acetylamino-22,23-dihydro avermectin B1b;
4"-deoxy-4"-dimethylamino avermectin B1a;
4"-deoxy-4"-dimethylamino avermectin B1b;
4"-deoxy-4"-dimethylamino-22,23-dihydro avermectin B1a;
4"-deoxy-4"-dimethylamino-22,23-dihydro avermectin B1b;
4"-deoxy-4"-p-chloro benzenesulfonylamino-22,23-dihydro avermectin B1a;
4"-deoxy-4"-p-chloro benzenesulfonylamino-22,23-dihydro avermectin B1b;
4"-deoxy-4"-(2-methylbenzenesulfonylamino)avermectin B1a;
4"-deoxy-4"-(2-methylbenzenesulfonylamino)avermectin B1b.

The "b" compounds, those with a 25-iso-propyl group, are very difficult to separate from the corresponding "a" compound with a 25-sec-butyl group and as such the compounds are generally isolated as mixtures of the two compounds. Thus references in the instant application to "a" compounds such as B1a, A1a, and the like, are construed to actually contain a certain proportion of the corresponding "b" compound. Alternatively, this representation of a mixture is sometimes done by referring to the B1 or B2 compounds or by separating the "a" compound from the "b" compound by a slash (/) such as B1a/B1b, B2a/B2b and the like.

The compounds of the instant invention are prepared using the procedure exemplified in the following reaction scheme:



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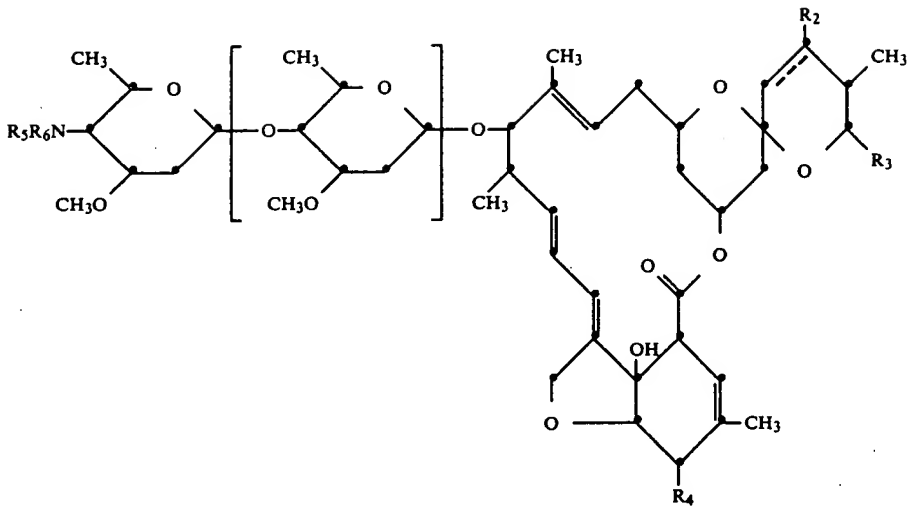


wherein R_2, R_3 and R_4 are as previously defined and the brackets indicate that the saccharide group contained therein may be either present or absent in the molecule.

In the first step of the foregoing reaction scheme, the avermectin starting materials which may be either the naturally occuring products, the 22,23-dihydro derivatives thereof or the monosaccharide derivative thereof, are oxidized at the 4"-position to the corresponding keto compound. During the procedure the presence of any hydroxy groups at the 5 and 23-position will require that such hydroxy groups be protected in order that they too are not oxidized. The 7-hydroxy group is very non-reactive and inert and need not be protected. The procedure used to prepare the protected intermediates are described below. The oxidation reaction is carried out in an inert solvent such as methylene chloride using oxalyl chloride or trifluoroacetic anhydride in dimethylsulfoxide as the oxidizing agent. Additionally N-chlorosuccinimide in dimethylsulfide may be employed. The reaction proceeds by dissolving the oxalyl chloride or trifluoroacetic anhydride and dimethylsulfoxide (or other oxidizing reagents) in methylene chloride and cooling to from −50° to −80° C. and adding dropwise a methylene chloride solution of the avermectin compound to be oxidized. The addition is carried out over a period of from 15 minutes to 1 hour and then triethylamine is added dropwise over a period of from 1 to 15 minutes. The reaction mixture is then allowed to warm to room temperature over a period of from ½ to 1 hour. The 4"-keto compound is isolated using techniques known to those skilled in the art.

In the next step, the 4"-keto compound is aminated to prepare the unsubstituted amino compound (R_5=R_6=hydrogen). The reaction is carried out in an inert solvent such as methanol at from −25° to +10° C. using ammonium salts and sodium cyanoborohydride as the aminating and reducing reagents. The reaction is complete in from 15 minutes to 2 hours and the product 4"-deoxy-4"-amino compound is isolated using techniques known to those skilled in the art. Suitable ammonium salts are the acetate, propionate, benzoate and the like. The acetate is preferred.

As a variation to the foregoing animation reaction, methyl ammonium salts could be used in place of the ammonium salts to prepare the monomethyl substituted compound directly. The same reagents, salts and reaction conditions as described above can be used for such a reaction.

The substitution reaction wherein the substituent is an acyl function is carried out using an acylating reagent in the presence of a base in an inert solvent. The preferred acylating reagents are loweralkanoyl anhydrides, loweralkanoyl halides, substituted benzene sulfonyl chlorides, lower alkyl sulfonyl chlorides, and the like. The reaction is carried out in an inert solvent such as methylene chloride in the presence of a non-reactive base such as pyridine or triethylamine in order to neutralize the acid produced during the course of the reaction. The reaction temperature is from −10° to 25° C. and the reaction is complete in from 5 minutes to 1 hour. The product is isolated using known techniques.

The reaction for the preparation of the 4"-deoxy-4"-dialkylamino compounds is carried out using the alkylating reaction conditions of formaldehyde and a reducing agent such as sodium borohydride, in methanol. The reaction is carried out in aqueous medium using excess aqueous formaldehyde along with the presence of a small amount of acid such as acetic acid to facilitate the reaction. The reaction is carried out at from −10° to +10° C. with the solution of the avermectin compound in methanol added dropwise over a period of from 30 to 60 minutes to the alkylating reagent mixture and the product is isolated using known techniques.

PREPARATION OF STARTING MATERIALS

Th ultimate starting materials for the compounds of this invention are the avermectin fermentation products defined above. Thus it is apparent that additional reactions are required to prepare the instant compounds. Specifically, reactions are carried out at the 5, 13, 22, and 23-positions. It is generally preferred to prepare whatever substituents are required at these positions before the oxidation at the 4"-hydroxy and subsequent substitution on the thus produced 4"-keto. Such a procedure generally avoids undesirable side reactions. This technique is not required, however, and if desired other sequences may be used. In addition, during the oxidation and substitution reaction described above, it is necessary to protect the hydroxy groups at the 5- and 23-positions to avoid oxidation or substitution at such positions. With these positions protected the reactions may be carried out at the 4-position without affecting the

remainder of the molecule. Subsequent to any of the above described reactions the protecting group may be removed and the unprotected product isolated. The protecting group employed is ideally one which may be readily synthesized, will not be affected by the reactions at the 4''-position and may be readily removed without affecting any other functions of the molecule. One preferred type of protecting group for the avermectin type of molecule is the tri-substituted silyl group, preferably the trialkyl silyl group. One especially preferred example, is the t-butyl dimethylsilyl group. The reaction preparing the protected compound is carried out by reacting the hydroxy compound with the appropriately substituted silylhalide, preferably the silylchloride in an aprotic polar solvent such as dimethylformamide. Imidazole is added as a catalyst. The reaction is complete in from 1 to 24 hours and at from 0° to 25° C. For the 5-position hydroxy group the reaction is complete in from ½ to 3 hours at from 0° C. to room temperature. This reaction is selective to the 5 position under the conditions above described and very little, if any, silylation is observed at other hydroxy substituted positions. If it is desired to protect the 23-hydroxy group a 4'', 5,23-tri(phenoxyacetyl) derivative can be prepared. Basic hydrolysis will leave the highly hindered 23-O-substituent but hydrolize the 5- and 4''-O-phenoxy acetyl groups. The 5-position is then protected as described above, selectively with t-butyldimethylsilyl.

The silyl group may be removed after the other contemplated reactions may be carried out. The silyl group or groups are removed by stirring the silyl compound in methanol catalized by a catalytic amount of an acid preferably a sulfonic acid such as p-toluene sulfonic acid. The reaction is complete in about 1 to 12 hours at from 0° to 50° C.

Another of the starting materials used in the foregoing reaction scheme are those in which the 22,23 double bond of the "1" type compounds has been reduced to a single bond. As is readily apparent from an analysis of the structure of avermectin starting materials there are 5 unsaturations in the l-series of compounds. Thus in the one series of compounds it is necessary to reduce the 22,23 double bond while not affecting the remaining four unsaturations or any other functional group present on the molecule in order to selectively prepare the 22,23 dihydro avermectins. It is necessary to select a specific catalyst for the hydrogenation, one that will selectively hydrogenate the least hindered from among a series of unsaturations. The preferred catalyst for such a selective hydrogenation procedure is one having the formula:

$$[(R_7)_3P)_3RhY)]$$

wherein R_7 is loweralkyl, phenyl or loweralkyl substituted phenyl and Y is halogen. The reduction procedure is completely described in U.S. Pat. No. 4,199,569.

The other starting materials which are used in the above reaction scheme involve the preparation of the mono-saccharide compound. That is those compounds wherein one of the α-1-oleandrosyl groups have been removed. The removal of the terminal α-1-oleandrose leaves a hydroxy group at the 4'-position which is equally amenable to the reactions described in the foregoing reaction scheme. Of course in such a case the products prepared are 4'-keto and 4'-deoxy 4'-amino derivatives rather than the 4''-keto and 4''-deoxy 4''-amino derivatives. The processes which may be used to prepare the monosaccharide derivatives of the avermectin compounds are described in U.S. Pat. No. 4,206,205. The reaction consists generally of treating the starting material disaccharide with acid in an aqueous organic solvent mixture. Water concentrations of from 0.1 to 20% by volume and acid concentrations of from about 0.01 to 0.1% will predominantly produce the monosaccharide product.

A further procedure for the preparation of the monosaccharide utilizes a 1% mineral acid solution in isopropanol at for 20°–40° C. preferably at room temperature for from 6 to 24 hours. Mineral acids such as sulfuric, hydrohalic, phosphoric and the like may be employed.

The novel compounds of this invention have significant parasiticidal activity as anthelmintics, ectoparasiticides, insecticides and acaricides, in human and animal health and in agriculture.

The disease or group of diseases described generally as helminthiasis is due to infection of an animal host with parasitic worms known as helminths. Helminthiasis is a prevalent and serious economic problem in domesticated animals such as swine, sheep, horses, cattle, goats, dogs, cats and poultry. Among the helminths, the group of worms described as nematodes causes widespread and often times serious infection in various species of animals. The most common genera of nematodes infecting the animals referred to above are Haemonchus, Trichostrongylus, Ostertagia, Nematodirus, Cooperia, Ascaris, Bunostomum, Oesophagostomum, Chabertia, Trichuris, Strongylus, Trichonema, Dictyocaulus, Capillaria, Heterakis, Toxocara, Ascaridia, Oxyuris, Ancylostoma, Uncinaria, Toxascaris and Parascaris. Certain of these, such as Nematodirus, Cooperia and Oesphagostomum attack primarily the intestinal tract while others, such as Haemonchus and Ostertagia, are more prevalent in the stomach while still others such as Dictyocaulus are found in the lungs. Still other parasites may be located in other tissues and organs of the body such as the heart and blood vessels, subcutaneous and lymphatic tissue and the like. The parasitic infections known as helminthiases lead to anemia, malnutrition, weakness, weight loss, severe damage to the walls of the intestinal tract and other tissues and organs and, if left untreated, may result in death of the infected host. The substituted avermectin compounds of this invention have unexpectedly high activity against these parasites, and in addition are also active against Dirofilaria in dogs, Namatospiroides, Syphacia, Aspiculuris in rodents, arthropod ectoparasites of animals and birds such as ticks, mites, lice, fleas, blowfly, in sheep Lucilia sp., biting insects and such migrating diperous larvae as Hypoderma sp. cattle, Gastrophilus in horses, and Cuterebra sp. in rodents.

The instant compounds are also useful against parasites which infect humans. The most common genera of parasties of the gastro-intestinal tract of man are Ancylostoma, Necator, Ascaris, Strongyloides, Trichinella, Capillaria, Trichuris, and Enterobius. Other medically important genera of parasites which are found in the blood or other tissues and organs outside the gastrointestinal tract are the filiarial worms such as Wuchereria, Brugia, Onchocerca and Loa, Dracunculus and extra intestinal stages of the intestinal worms Strongyloides and Trichinella. The compounds are also of value against arthropods parasitizing man, biting insects and other dipterous pests causing annoyance to man.

The compounds are also active against household pests such as the cockroach, *Blatella sp.*, clothes moth,

Tineola sp., carpet beetle, Attagenus sp., and the housefly *Musca domestica.*

The compounds are also useful against insect pests of stored grains such as Tribolium sp., Tenebrio sp. and of agricultural plants such as spider mites, (Tetranychus sp.), aphids, (Acyrthiosiphon sp.); against migratory orthopterans such as locusts and immature stages of insects living on plant tissue. The compounds are useful as a nematocide for the control of soil nematodes and plant parasites such as Meloidogyne spp. which may be of importance in agriculture. The compoundss are active against other plant pests such as the southern army worm and Mexican bean beetle larvae.

These compounds may be administered orally in a unit dosage form such as a capsule, bolus or tablet, or as a liquid drench where used as an anthelmintic in mammals. The drench is normally a solution, suspension or dispersion of the active ingredient usually in water together with a suspending agent such as bentonite and a wetting agent or like excipient. Generally, the drenches also contain an antifoaming agent. Drench formulations generally contains from about 0.001 to 0.5% by weight of the active compound. Preferred drench formulations may contain from 0.01 to 0.1% by weight. The capsules and boluses comprise the active ingredient admixed with a carrier vehicle such as starch, talc, magnesium stearate, or di-calcium phosphate.

Where it is desired to administer the avermectin derivatives in a dry, solid unit dosage form, capsules, boluses or tablets containing the desired amount of active compound usually are employed. These dosage forms are prepared by intimately and uniformly mixing the active ingredient with suitable finely divided diluents, fillers, disintegrating agents and/or binders such as starch, lactose, talc, magnesium stearate, vegetable gums and the like. Such unit dosage formulations may be varied widely with respect to their total weight and content of the antiparasitic agent depending upon factors such as the type of host animal to be treated, the severity and type of infection and the weight of the host.

When the active compound is to be administered via an animal feedstuff, it is intimately dispersed in the feed or used as a top dressing or in the form of pellets which may then be added to the finished feed or optionally fed separately. Alternatively, the antiparasitic compounds of our invention may be administered to animals parenterally, for example, by intraruminal, intramuscular, intratracheal, or subcutaneous injection in which event the active ingredient is dissolved or dispersed in a liquid carrier vehicle. For parenteral administration, the active material is suitably admixed with an acceptable vehicle, preferably of the vegetable oil variety such as peanut oil, cotton seed oil and the like. Other parenteral vehicles such as organic preparation using solketal, glycerol formal, and aqueous parenteral formulations are also used. The active avermectin compound or compounds are dissolved or suspended in the parenteral formulation for administration; such formulations generally contain from 0.005 to 5% by weight of the active compound.

Although the antiparasitic agents of this invention find their primary use in the treatment and/or prevention of helminthiasis, they are also useful in the prevention and treatment of diseases caused by other parasites, for example, arthropod parasites such as ticks, lice, fleas, mites and other biting insects in domesticated animals and poultry. They are also effective in treatment of parasitic diseases that occur in other animals including humans. The optimum amount to be employed for best results will, of course, depend upon the particular compound employed, the species of animal to be treated and the type and severity of parasitic infection or infestation. Generally good results are obtained with our novel compounds by the oral administration of from about 0.001 to 10 mg per kg of animal body weight, such total dose being given at one time or in divided doses over a relatively short period of time such as 1-5 days. With the preferred compounds of the invention, excellent control of such parasties is obtained in animals by administering from about 0.025 to 0.5 mg per kg of body weight in a single dose. Repeat treatments are given as required to combat re-infections and are dependent upon the species of parasite and the husbandry techniques being employed. The techniques for adminstering these materials to animals are known to those skilled in the veterinary field.

When the compounds described herein are administered as a component of the feed of the animals, or dissolved or suspended in the drinking water, compositions are provided in which the active compound or compounds are intimately dispersed in an inert carrier or diluent. By inert carrier is meant one that will not react with the antiparasitic agent and one that may be administered safely to animals. Preferably, a carrier for feed administration is one that is, or may be, an ingredient of the animal ration.

Suitable compositions include feed premixes or supplements in which the active ingredient is present in relatively large amounts and which are suitable for direct feeding to the animal or for addition to the feed either directly or after an intermediate dilution or blending step. Typical carriers or diluents suitable for such compositions include, for example, distillers' dried grains, corn meal, citrus meal, fermentation residues, ground oyster shells, wheat shorts, molasses solubles, corn cob meal, edible bean mill feed, soya grits, crushed limestone and the like. The active hydrogenated avermectin compounds are intimately dispersed throughout the carrier by methods such as grinding, stirring, milling or tumbling. Compositions containing from about 0.005 to 2.0% by weight of the active compound are particualrly suitable as feed premixes. Feed supplements, which are fed directly to the animal, contain from about 0.0002 to 0.3% by weight of the active compounds.

Such supplements are added to the animal feed in an amount to give the finished feed the concentration of active compound desired for the treatment and control of parastic diseases. Although the desired concentration of active compound will vary depending upon the factors previously mentioned as well as upon the particular avermectin derivative employed, the compounds of this invention are usually fed at concentrations of between 0.00001 to 0.002% in the feed in order to achieve the desired antiparasitic result.

The avermectin compounds of this invention are also useful in combatting agricultural pests that inflict damage upon crops while they are growing or while in storage. The compounds are applied using known techniques as sprays, dusts, emulsions and the like, to the growing or stored crops to effect protection from such agricultural pests.

In using the compounds of this invention, the individual substituted avermectin components may be prepared and used in that form. Alternatively, mixtures of two or more of the individual avermectin components

may be used, as well as mixtures of the parent avermectin compounds, other avermectin compounds or other active compounds not related to avermectin, with the compounds of this invention.

The 4''-amino and substituted amino compounds of the present invention are valuable antibiotics active against various Gram-positive and Gram-negative bacteria and accordingly find utility in human and veterinary medicine. Representative pathogens which are sensitive to the instant compounds include: *Staphylococcus aureus, Escherichia coli, Klebsiella pneumoniae, Bacillus subtilis, Salmonella typhosa, Psuedomonas* and *Bacterium proteus.* The antibacterials of the invention are not limited to utility as medicaments; they may be used in all manner of industry, for example: additives to animal feed, preservation of food, disinfectants, and in other industrial systems where control of bacterial growth is desired. For example, they may be employed in aqueous compositions in concentrations ranging from 0.1 to 100 parts of antibiotic per million parts of solution in order to destroy or inhibit the growth of harmful bacteria on medical and dental equipment and as bactericides in industrial applications, for example in waterbased paints and in the white water of paper mills to inhibit the growth of harmful bacteria.

The products of this invention may be used in any of a variety of pharmaceutical preparations. They may be employed in capsule, powder form, in liquid solution, or in suspension. They may be administered by a variety of means; those of principal interest include: orally, topically or parenterally by injection (intravenously or intramuscularly).

Such tablets and capsules, designed for oral administration, may be in unit dosage form, and may contain conventional excipients, such as binding agents, for example, syrup, acacia, gelatin, sorbitol, tragacanth, or polyvinylpyrrolidone; fillers, for example, lactose, sugar, cornstarch, calcium phosphate, sorbitol, or glycerine; lubricants, for example, magnesium stearate, talc, polyethylene glycol, silica; disintegrants, for example, potato starch, acceptable wetting agents such as sodium lauryl sulphate. The tablets may be coated according to methods well known in the art. Oral liquid preparations may be in the form of aqueous or oily suspensions, or solutions, or they may be presented as a dry product for reconstitution with water or other suitable vehicle before use. Such liquid preparations may contain conventional additives such as suspending agents, for example, sorbitol, methyl cellulose, glucose/sugar syrup, gelatin, hydroxyethylcellulose, or carboxymethyl cellulose. Suppositories will contain conventional suppository bases, such as cocoa butter or other glycerides.

Composition for injection, the preferred route of delivery, may be prepared in unit dosage form in ampules, or in multidose containers. The compositions may take such forms as suspensions, solutions, or emulsions in oily or aqueous vehicles, and may contain formulatory agents. Alternatively, the active ingredient may be in powder form for reconstitution, at the time of delivery, with a suitable vehicle, such as sterile water.

The compositions may also be prepared in suitable forms for absorption through the mucous membranes of the nose and throat or bronchial tissues and may conveniently take the form of liquid sprays or inhalants, lozenges, or throat paints. For medication of the eyes or ears, the preparation may be presented in liquid or semisolid form. Topical applications may be formulated in hydrophobic or hydrophilic bases as ointments, creams, lotions, paints, or powders.

The dosage to be administered depends to a large extent upon the condition and size of the subject being treated as well as the route and frequency of administration—the parenteral route by injection being preferred for generalized infections. Such matters, however, are left to the routine discretion of the therapist according to principles of treatment well known in the antibiotic art. In general, a daily dosage consists of from about 0.1 to about 5 mg of active ingredient per kg. of body weight of the subject in one or more treatments per day. A preferred daily dosage for adult humans lies in the range of from about 0.1 to 20 mg. of active ingredient per kg. of body weight. Another factor influencing the precise dosage regimen, apart from the nature of the infection and peculiar identity of the individual being treated, is the molecular weight of the carbon species of this invention.

The compositions for human delivery per unit dosage, whether liquid or solid, may contain from 0.1% to 99% of active material, the preferred range being from about 10–60%. The composition will generally contain from about 5 mg. to about 50 mg. of the active ingredient; however, in general, it is preferable to employ a dosage amount in the range of from about 5 mg to 100 mg. In parenteral administration, the unit dosage is usually the pure compound I in sterile water solution or in the form of a soluble powder intended for solution.

In the isolation of the avermectin compounds, which serve as starting materials for the instant process, from the fermentation broth, the various avermectin compounds will be found to have been prepared in unequal amounts. In particular an "a" series compound will be prepared in a higher proportion than the corresponding "b" series compound. The difference between the "a" series and "b" series is constant throughout the avermectin compounds and consists of a sec-butyl group and an iso-propyl group respectively at the 25 position. This difference, of course, does not interfere with any of the instant reactions. In particular it may not be necessary to separate the "b" components from the related "a" component. Separation of these closely related compounds is generally not practiced since the "b" compound is present only in a very small percent by weight, and the structural difference has negligible effect on the reaction processes and biological activities.

In particular it has been found that the starting materials for the compounds of this invention are very often prepared in a ratio of about 80% avermectin B1a and A1a and 20% avermectin B1b or A1b. Thus the preferred composition of this invention is one which contains about 80% of the "a" component and 20% of the "b" component.

The following examples are provided in order that this invention might be more fully understood; they are not to be construed as limitative of the invention.

The substituted avermectin derivatives prepared in the following examples are generally isolated as amorphous solids and not as crystalline solids. They are thus characterized analytically using techniques such as mass spectrometry, nuclear magnetic resonance, and the like. Being amorphous, the compounds are not characterized by sharp melting points, however, the chromatographic and analytical methods employed indicate that the compounds are pure.

In the following examples, the various starting materials therefor are avermectin compounds or derivatives

of avermectin compounds. The avermectin compounds and the preparation and isolation thereof from fermentation broths are described in U.S. Pat. No. 4,310,519 issued Jan. 12, 1982. The selective 22,23-dihydro derivatives of avermectin compounds are decribed in U.S. Pat. No. 4,199,569 issued Apr. 22, 1980. The monosaccharide derivatives of avermectin compounds are described in U.S. Pat. No. 4,206,205 issued Jan. 3, 1980.

EXAMPLE 1

5-O-t-butyl-dimethylsilyl-22,23-dihydro avermectin B1a/B1b

3 g of 22,23-dihydro avermectin B1a/B1b in 30 ml of dry dimethylformamide was combined with 1.4 g of imidazole and stirred at room temperature until all the materials had dissolved. Then 1.56 g of t-butyl-dimethylsilyl chloride was added and the reaction mixture stirred at room temperature for 70 minutes. The reaction mixture was diluted with 150 ml of ether, water was added and the layers were separated. The aqueous layer was extracted twice more with ether and the combined ether layers washed four times with water and once with saturated sodium chloride solution. The ether layer was dried over magnesium sulfate and concentrated to dryness in vacuo affording 4.2 g of a white foam. The foam is chromatographed on 135 g. of 70–230 mesh silica gel and eluted with 5% tetrahydrofuran in methylene chloride. 1.15 G of 4"-5-di-O-t-butyl-dimethylsilyl-22,23-dihydro avermectin B1a/B1b and 2.6 g of 5-O-t-butyl dimethylsilyl-22,23-dihydro avermectin B1a/B1b were recovered as pure amorphous foams.

EXAMPLE 2

5-O-t-butyl-dimethylsilyl-4"-keto-22,23-dihydro-avmerectin B1a/B1b

In a dried flask purged with dry nitrogen was placed 97 μl of oxalyl chloride and 1.5 ml of methylene chloride. The reaction mixture was cooled to −60° C., 1 ml of the methylene chloride solution containing 160 μl of dimethylsulfoxide was added over a period of 3 minutes and the reaction mixture stirred at −60° C. for two minutes. 3 Ml of methylene chloride containing 500 mg of 5-O-t-butyl-dimethylsilyl 22,23-dihydro avermectin B1a/B1b was added dropwise over a period of 5 minutes and the reaction mixture stirred at room temperature for 30 minutes. At the end of this period, 0.71 ml of triethylamine was added dropwise and the reaction mixture was stirred at −60° C. for 5 minutes. The cold bath was removed and the reaction mixture was allowed to come to room temperature over a period of 45 minutes. 50 Ml of water was added and the reaction mixture was extracted 3 times with 40 ml of ether. The ether extracts were combined and washed 4 times with 20 ml of water, dried over magnesium sulfate and concentrated to dryness in vacuo affording 520 mg of a yellow glass. The yellow glass was dissolved in methylene chloride and placed on three 2,000μ silica gel preparative layer chromatography plates. The plates were developed with 10% ethyl acetate in methylenechloride and afforded 470 ml of yellow foam which was characterized by its 300 MHz nuclear magnetic resonance spectrum as 5-O-t-butyl-dimethylsilyl-4"-keto-22,23-dihydro avermectin B1a/B1b.

EXAMPLE 3

5-O-t-butyl-dimethylsilyl-4"-deoxy-4"-amino-22,23-dihydro avermectin B1a/B1b

Into a dried flask was placed 200 mg of 5-O-t-butyl-dimethylsilyl-4"-keto-22,23-dihydro-avermectin B1a/B1b 2 ml of methanol, 160 mg of ammonium acetate and 12 mg of sodium cyanoborohydride. The reaction mixture was stopped and stirred at room temperature for 100 minutes. The reaction mixture was added to a 10 ml solution of saturated sodium carbonate diluted 1 to 1 with water to afford a gummy precipitate. The precipitate was extracted 4 times with 3 ml of ethyl acetate and the ethyl acetate layers were combined and washed four times with 1 ml of water. The organic layer was dried over magnesium sulfate and evaporated to dryness in a stream of nitrogen to afford 198 mg of yellow opaque glass. The glass was dissolved in methylene chloride and placed on three 1,000μ silica gel preparative layer chromatography plates and eluted with 7% methanol in methylene chloride to afford 19 mg of a yellow glass which was identified by 300 MHz nuclear magnetic resonance as 5-O-t-butyl-dimethylsilyl-4"-deoxy-4"-amino-22,23-dihydro avermectin B1a/B1b and 64 mg of a yellow glass which was identified by 300 MHz nuclear magnetic resonance as 5-O-t-butyl-dimethylsilyl-4"-deoxy-4"-epiamino-22,23-dihydro avermectin B1b/B1b.

EXAMPLE 4

4"-deoxy-4"-amino-22,23-dihydro-avermectin B1a/B1b

17 mg of 5-O t-butyl-dimethylsilyl -4"deoxy-4"-amino-22,23-dihydro avermectin B1a/B1b was dissolved in a few drops of methanol and cooled in an ice-bath with stirring. 3 ml of 1% (weight to volume), p-toluene sulfonic acid hydrate solution in methanol was added and the reaction mixture stirred for 55 minutes. The reaction mixture was added to dilute sodium bicarbonate solution and extracted 3 times with 3 ml portions of ethyl acetate. The organic layers were combined and washed 3 times with 1 ml of water and once with saturated sodium chloride solution. The organic layer was dried over magnesium sulfate and evaporated to dryness under a stream of nitrogen affording 18 mg of a yellow glass. The yellow glass was dissolved in methylene chloride and placed on a 250μ silica gel preparative layer chromatography plate and eluted with 8% methanol in methylene chloride. The band with an Rf of 0.20 afforded 8.5 mg of a white glass. Mass spectrometry and 300 MHz nuclear magnetic resonance identified it as 4"-deoxy-4"-amino-22,23-dihydro-avermectin B1a/B1b.

EXAMPLE 5

4"-deoxy-4"-epi-amino-22,23-dihydro avermectin B1a/B1b

Following the procedure of Example 4 using 60 mg of 5-O-t-butyl-dimethylsilyl 4"-deoxy-4" epi-amino-22,23-dihydro avermectin B1a/B1b 6.0 ml of 1% weight to volume p-toluene sulfonic acid hydrate in methanol there was obtained 27.4 mg of 4"-deoxy-4"-epi-amino-22,23-dihydro avermectin B1a/B1b which structure was confirmed by mass spectrometry and 300 MHz nucelar magnetic resonance.

EXAMPLE 6

5-O-t-butyl-dimethylsilyl-4''-deoxy-4''-acetylamino 22,23-dihydro avermectin Bla/Blb

30 mg of 5-O-t-butyl-dimethylsilyl-4'' deoxy-4'' amino-22,23-dihydro avermectin Bla/Blb was combined with 0.5 ml of methylene chloride and cooled in an ice-bath. 6 Drops of pyridene was added followed by 2 Drops of acetic anhydride. The reaction mixture was stirred at 0° C. for 30 minutes. Water and ether were added, the layers were separated and the aqueous layer extracted again with ether. The organic layers were combined and washed four times with 1 ml portions of water, dried over magnesium sulfate and evaporated to dryness under a stream of nitrogen to afford 35 mg of a yellow glass. The yellow glass was dissolved in methylene chloride and placed on a 500μ silica gel preparative layer chromatography plate and eluted with a 1 to 1 mixture of ethyl acetate in methylene chloride to afford 14.6 mg of off-white foam. 300 MHz nuclear magnetic resonance characterized the material is 5-O-t-butyldimethylsilyl-4''-deoxy-4''-acetylamino-22,23-dihydro-avermectin Bla/Blb.

EXAMPLE 7

4''-deoxy-4''-acetylamino-22,23-dihydro avermectin Bla/Blb

Following the procedure of Example 4, 4''-acetylamino-4''-deoxy-5-O-t-butyldimethylsilyl-22,23-dihydroavermectin Bla/Blb was treated with a 1% solution of p-toluenesulfonic acid monohydrate in methanol (w/v) to give after isolation and purification 4''-acetylamino-4''-deoxy-22,23-dihydroavermectin Bla/Blb.

EXAMPLE 8

5-O-t-butyl-dimethylsilyl-4''-deoxy-4''-epi-acetylamino-22,23-dihydro-avermectin Bla/Blb

Following the procedure of Example 6 wherein 5 mg of 5-O-t-butyl-dimethylsilyl-4''-deoxy-4''-epi-amino-22,23-dihydro avermectin Bla/Blb was employed, there was obtained 5-O-t-butyl-dimethylsilyl 4''-deoxy-4''-epi-acetylamino-22,23-dihydro avermectin Bla/Blb.

EXAMPLE 9

4''-deoxy-4''-epi-acetylamino-22,23-dihydro-avermectin Bla/Blb

Following the procedure of Example 4 in which 35 mg of 5-O-t-butyl-dimethylsilyl-4''-deoxy-4''-epi-acetylamino-22,23-dihydro avermectin Bla/Blb was employed with 4 ml of 1% weight to volume p-toluene sulfonic acid hydrate in methanol. There was obtained 19.4 mg of 4''-deoxy-4''-epi-acetylamino-22,23-dihydro-avermectin Bla/Blb.

EXAMPLE 10

4''-keto-22,23-dihydro-avermecting Bla/Blb

25 mg of 5-O-t-butyldimethylsilyl-4''-keto-22,23-dihydro-avermectin Bla/Blb in 3 mg of 1% weight to volume p-toluene sulfonic acid hydrate in methanol was stirred at room temperature for 20 minutes. 20 Ml of water was added and the reaction mixture extracted three times with 15 ml portions of ether. The ether layers were combined and washed three times with 3 ml portions of water dried over magnesium sulfate and evaporated to dryness in vacuo to afford 15 mg of a colorless glass. The glass was dissolved in methylene chloride and placed on a 1000μ silica gel preparative layer chromatography plate and eluted with 5% methanol in methylene chloride to afford 14.5 mg of an off-white glass characterized by mass spectrometry and 300 MHz nuclear magnetic resonance as 4''-keto 22,23-dihydro-avermectin Bla/Blb.

EXAMPLE 11

5-O-t-butyldimethylsilyl-4''-deoxy-4''-epi-N,N-dimethylamino-22,23-dihydro avermectin Bla/Blb

50 mg of 5-O-t-butyl-dimethylsilyl-4''-deoxy-4''-epi-amino-22,23-dihydro-avermectin Bla/Blb was dissolved in 0.7 ml of methanol and 0.4 ml of acetic acid added followed by 0.5 ml of 37% aqueous formaldehyde solution. The reaction mixture was stirred at room temperature for 30 minutes, cooled in an ice bath and 130 mg of sodium borohydride was added in 5–10 mg portions with 5–10 minutes separating each portion. Vigorous foaming accompanied each addition. A saturated sodium bicarbonate solution was added in small portions to the reaction mixture to neutrality followed by extraction with ether. The aqueous layer was extracted twice more with 20 ml portions of ether. The ether layers were combined and washed four times with 4 ml portions of water. The combined organic layers were dried over magnesium sulfate and evaporated to dryness in vacuo affording 40 ml of a slightly yellow glass. The glass was dissolved in methylene chloride and placed on a 1000μ silica gel preparative layer chromatography plate and developed with ethyl acetate to afford 30.5 mg of yellow glass identified by mass spectrometry as 5-O-t-butyldimethylsilyl-4''-deoxy-4''-epi-N,N-dimethylamino-22,23-dihydro avermectin Bla/Blb.

EXAMPLE 12

4''-deoxy-4''-epi-N,N-dimethylamino-22,23-dihydro-avermectin Bla/Blb

29 Mg of 5-O-t-butyl-dimethylsilyl-4''-deoxy-4''-epi-N,N-dimethylamino-22,23-dihydro avermectin Bla/Blb was treated with 3 ml of 1% weight to volume p-toluene sulfonic acid hydrate in methanol following the procedure of Example 4. There was afforded 15.8 mg of a white glass which was identified by mass spectrometry and 300 MHz nuclear magnetic resonance as 4''-deoxy-4''-epi-N,N-dimethylamino-22,23-dihydro-avermectin Bla/Blb.

EXAMPLE 13

5-O-t-Butyldimethylsilyl-4''-deoxy-4''-(4-chlorophenylsulfonylamino)-22,23-dihydroavermectin-Bla/Blb

A solution of 100 mg of 5-O-t-butyldimethylsilyl-4''-deoxy-4''-amino-22,23-dihydroavermectin Bla/Blb is dissolved in 2 ml of methylenechloride and treated with 70 mg of triethylamine and 45 mg of 4-chlorobenzenesulfonylchloride at room temperature for 16 hrs. The usual work-up gives 5-O-t-butyldimethylsilyl-4''-deoxy-4''-(4-chlorophenylsulfonylamino)-22,23-dihydroavermectin-Bla/Blb in pure form.

EXAMPLE 14

4''-Deoxy-4''-(4-chlorophenylsulfonylamino)-22,23-dihydroavermectin-Bla/Blb

100 Mg of 5-O-t-butyldimethylsilyl-4''-deoxy-4''-(4-chlorophenylsulfonylamino)-22,23-dihydroavermectin-Bla/Blb is treated according to Example 4 with a solu-

tion of 1% of p-toluenesulfonic acid monohydrate in methanol for 30 min at room temperature affording 4''-deoxy-4''-(4-chlorophenylsulfonylamino)-22,23-dihydroavermectin-Bla/Blb in pure form.

EXAMPLE 15

4''-Keto-5-O-t-butyldimethylsilylavermectin-Bla/Blb

If avermectin Bla/Blb is reacted according to the procedures of Examples 1 and 2 4''-keto-5-O-t-butyldimethylsilylavermectin-Bla/Blb is obtained.

EXAMPLE 16

4''-Keto-avermectin-Bla/Blb

If the product of Example 15 is reacted according to the procedure of Example 10, 4''-ketoavermectin-Bla/Blb is obtained.

EXAMPLE 17

4''-Amino-4''-deoxyavermectin Bla/Blb

If the product of Example 15 is reacted according to the procedures of Examples 3 and 4 4''-amino-4''-deoxyavermectin Bla/Blb is obtained.

EXAMPLE 18

4''-Acetylamino-4''-deoxyavermectin Bla/Blb

If the product of Example 15 is reacted according to the procedures of Examples 3, 6 and 7 4''-acetylamino-4''-deoxavermectin Bla/Blb is obtained.

EXAMPLE 19

4'-Keto-5-O-t-butyldimethylsilyl-22,23-dihydroavermectin Bla/Blb monosaccharide

If 22,23-dihydroavermectin Bla/Blb-monosaccharide is reacted according to the procedures of Examples 1 and 2, 4'-keto-5-O-t-butyldimethylsilyl-22,23-dihydroavermectin Bla/Blb monosaccharide is obtained.

EXAMPLE 20

4'-Keto-22,23-dihydroavermectin Bla/Blb-monosaccharide

If the product of Example 19 is reacted according to the procedures of Example 10 4'-keto-22,23-dihydroavermectin Bla/Blb-monosaccharide is obtained.

EXAMPLE 21

4'-Amino-4'-deoxy-22,23-dihydroavermectin-Bla/Blb monosaccharide

If the product of Example 19 is reacted according to the procedures of Examples 3 and 4, 4'-amino-4'-deoxy-22,23-dihydroavermectin-Bla/Blb-monosaccharide is obtained.

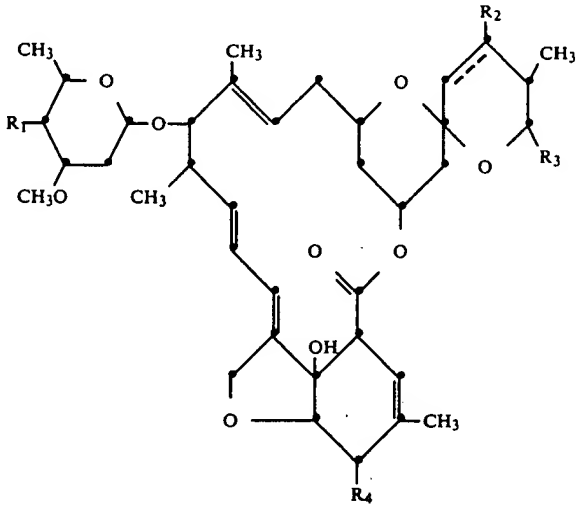
EXAMPLE 22

4'-Acetylamino-4'-deoxy-22,23-dihydroavermectin-Bla/Blb-monosaccharide

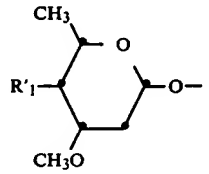
If the product of Example 19 is reacted according to the procedures of Examples 3, 6 and 7, 4'-acetylamino-4'-deoxy-22,23-dihydroavermectin-Bla/Blb-monosaccharide is obtained.

What is claimed is:

1. A compound having the formula:



wherein R_1 is =O, $-NR_5R_6$ or



wherein

R_1' is =O or $-NR_5R_6$:

R_5 and R_6 are independently hydrogen, loweralkyl, loweralkanoyl, substituted benzenesulfonyl wherein the substituent is halogen; or loweralkyl sulfonyl;

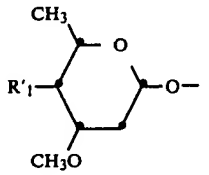
R_2 is hydrogen or hydroxy;

R_3 is sec-butyl or iso-propyl;

R_4 is hydroxy or methoxy;

and the broken line indicates a single or a double bond at the 22,23-position provided that R_2 can only be hydroxy when the broken line indicates a single bond.

2. The compound of claim 1 wherein R_1 is:



and R'_1 is =O, or $-NR_5R_6$ wherein R_5 and R_6 are independently hydrogen, methyl or acetyl.

3. The compound of claim 2 wherein R'_1 is =O.

4. The compound of claim 3 which is 4''-keto-avermectin Bla/Blb.

5. The compound of claim 3 which is 4''-keto-22,23-dihydro avermectin Bla/Blb.

6. The compound of claim 2 wherein R'_1 is $-NR_5R_6$ and R_5 and R_6 are independently hydrogen, methyl or acetyl.

7. The compound of claim 6 which is 4''-deoxy-4''-amino-avermectin Bla or Blb.

8. The compound of claim 6 which is 4''-deoxy-4''-amino-22,23-dihydro-avermectin Bla or Blb.

9. The compound of claim 6 which is 4''-deoxy-4''-acetylamino-avermectin Bla or Blb.

10. The compound of claim 6 which is 4''-deoxy-4''-acetylamino-22,23-dihydro avermectin Bla or Blb.

11. The compound of claim 6 which is 4''-deoxy-4''-N,N-dimethylamino-avermectin Bla or Blb.

12. The compound of claim 6 which is 4''-deoxy-4''-N,N-dimethylamino-22,23-dihydro avermectin Bla or Blb.

13. A method for the treatment of helminthiasis which comprises administering to an animal infected with helminths an effective amount of a compound of claim 1.

14. A composition useful for treating animals infected with helminths which comprises an inert carrier and an effective amount of a compound of claim 1.

* * * * *

ATTACHMENT B

UNITED STATES DEPARTMENT OF COMMERCE
Patent and Trademark Office
Address: COMMISSIONER OF PATENTS AND TRADEMARKS
Washington, D. C. 20231

PAYOR NUMBER
000210

75M3/0728

MERCK & CO., INC.
PATENT DEPARTMENT
P.O. BOX 2000
RAHWAY, NJ 07065-0907

MAINTENANCE FEE STATEMENT

The data shown below is from the records of the Patent and Trademark Office. If the maintenance fees and any necessary surcharges have been timely paid for the patents listed below, the notation "PAID" will appear in column 10, "status" below.

If a maintenance fee payment is defective, the reason is indicated by code in column 10, "status" below. An explanation of the codes appears on the reverse of the Maintenance Fee Statement. TIMELY CORRECTION IS REQUIRED IN ORDER TO AVOID EXPIRATION OF THE PATENT. NOTE 37 CFR 1.377. THE PAYMENT(S) WILL BE ENTERED UPON RECEIPT OF ACCEPTABLE CORRECTION. IF PAYMENT OR CORRECTION IS SUBMITTED DURING THE GRACE PERIOD, A SURCHARGE IS ALSO REQUIRED. NOTE 37 CFR 1.20(k) and (l).

If the statement of small entity status is defective the reason is indicated below in column 10 for the related patent number. THE STATEMENT OF SMALL ENTITY STATUS WILL BE ENTERED UPON RECEIPT OF ACCEPTABLE CORRECTION.

ITM NBR	PATENT NUMBER	FEE CDE	FEE AMOUNT	SUR CHARGE	SERIAL NUMBER	PATENT DATE	FILE DATE	PAY YR	SML ENT	STAT
1	4,427,663	185	2900	----	06/358,736	01/24/84	03/16/82	12	NO	PAID

If the "status" column for a patent number listed above does not indicate "PAID" a code or an asterisk (*) will appear in the "status" column. Where an asterisk (*) appears, the codes are set out below by the related item number. An explanation of the codes indicated in the "status" column and as set out below by the related item number appears on the reverse of the maintenance fee statement.

ITM NBR	ATTY DKT NUMBER
1	16568

**DIRECT THE RESPONSE TOGETHER WITH ANY QUESTIONS ABOUT THIS NOTICE TO:
COMMISSIONER OF PATENTS AND TRADEMARKS, BOX M. FEE, WASHINGTON, DC 20231**

ATTACHMENT C

Sponsor Courtesy Copy

[illegible] 16 1997

FREEDOM OF INFORMATION SUMMARY

ORIGINAL NEW ANIMAL DRUG APPLICATION

NADA 141-079

IVOMEC® EPRINEX™ (eprinomectin) Pour-On for Beef and Dairy Cattle

Sponsored by

MERCK & CO., INC.

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I. GENERAL INFORMATION

NADA Number: 141-079

Sponsor: Merck Research Laboratories
Division of Merck & Co., Inc.
P. O. Box 2000
Rahway, New Jersey 07065-0914

Established Name: Eprinomectin

Trade Name: IVOMEC® EPRINEX™ Pour-On for Beef and Dairy Cattle

Marketing Status: Over-the-counter

II. INDICATIONS FOR USE

IVOMEC EPRINEX Pour-On for Beef and Dairy Cattle is indicated for treatment and control of:

Gastrointestinal nematodes (adults and fourth-stage larvae, L_4)
Haemonchus placei
Ostertagia ostertagi (including inhibited L_4)
Trichostrongylus axei
Trichostrongylus colubriformis
Cooperia oncophora
Cooperia punctata
Cooperia surnabada
Nematodirus helvetianus
Bunostomum phlebotomum
Oesophagostomum radiatum
Trichuris spp. (adults)

Lungworms (adults and L_4)
Dictyocaulus viviparus

Cattle grubs (all parasitic stages)
Hypoderma lineatum
Hypoderma bovis

Lice
Damalinia bovis
Linognathus vituli
Haematopinus eurysternus
Solenopotes capillatus

Mange Mites
Chorioptes bovis
Sarcoptes scabiei

Flies
Haematobia irritans

IVOMEC EPRINEX (eprinomectin) Pour-On for Beef and Dairy Cattle has been proved to control infections of *Dictyocaulus viviparus* for 21 days after treatment and *Haematobia irritans* for 7 days after treatment.

III. DOSAGE FORM, ROUTE OF ADMINISTRATION AND DOSAGE

A. **Dosage Form**: IVOMEC EPRINEX Pour-On is a clear non-aqueous solution containing 5 mg per ml of eprinomectin. The product is available in 250 ml, 1 liter, 2.5 liter and 5 liter plastic bottles.

B. **Route of Administration**: IVOMEC EPRINEX Pour-On should be applied topically along the backline from the withers to the tailhead.

C. **Approved Dose**: The recommended dose of IVOMEC EPRINEX Pour-On is 1 ml per 10 kg body weight to deliver 500 mcg per kg body weight of eprinomectin.

l. **No-Observed-Effect Level:** 2 mg/kg/day for maternal toxicity and > 8 mg/kg/day for embryo/fetal viability.

11. Multigeneration Study in Rats

a. **Report Number:** TT #90-9010.

b. **Study Dates:** Started 22JUN90, ended 20JUN91.

c. **Principal Investigators:** A. Brooker, D. Myers, C. Parker.

d. **Laboratory:** Huntingdon Research Centre Ltd., Huntingdon, Cambridgeshire, England.

e. **Substance and Dosage Form Tested:** MK-0397 (L-653,648-000X014)

f. **Species and Strain:** Rat/Sprague-Dawley (Crl:CD®(SD)BR).

g. **Number of Animals/Sex/Group:** 32/sex/group for F_0 generation; 28/sex/group and 24/sex/group for F_1 and F_2 generations, respectively.

h. **Dosage Levels Tested:** 0, 6, 18, and 54 ppm (equivalent to approximately 0.5, 1.5, and 4.5 mg/kg/day).

i. **Route of Administration:** Oral via diet.

j. **Parameters Examined:** Physical signs, food consumption and body weights were recorded weekly. Water consumption was measured daily over the initial and final two weeks of pre-mating for each generation. Reproductive parameters assessed included mating performance, fertility index, numbers of pups/litter, pup weights and sexual maturation of pups. Histologic examination of the reproductive tract was conducted for the F_0 and F_1 high dose and control group males and females and target organs and gross lesions from all animals.

k. **Toxicity Observed:** Increases in body weight gain and food and water consumption were found in the high dose group F_0 animals. Decreased mating performance was also evident in the high dose group. Neonatal toxicity characterized by increased pup mortality, tremors, and decreased pup weights were found in the high dose F_1 and F_2 pups, while toxicity in the mid dose F_1 pups was limited to tremors in a few pups. Due to marked increases in food consumption during lactation resulting in increases in drug intake in the F_0 and F_1 animals, the F_1 animals were re-mated and the diet concentrations of drug reduced by a factor of 2 to maintain more constant drug intake values. As a result, drug intake values were approximately 0.4, 1.3, and 3.3 mg/kg/day during lactation of the F_{2b} offspring, compared to values of 1.0, 3.0, and 6.5 mg/kg/day for the F_{2a} offspring. In the F_{2b} offspring tremors were again noted in the high dose group pups. However, no toxicity was found in the mid and low dose group pups

l. **No-Observed-Effect Level:** 1.0 to 1.5 mg/kg/day.

B. Safe Concentrations of Total Residues

The most appropriate toxicity study for determining the safe concentrations for eprinomectin-related residues in milk and edible tissues is the 53-week oral toxicity study in dogs. The no-observed-effect level (NOEL) for this study is 1.0 mg/kg/day. The Acceptable Daily Intake (ADI) based on a NOEL of 1.0 mg/kg/day and a safety factor of 100 is 10 mcg/kg/day, calculated as follows:

ADI = 1.0 mg/kg/day ÷ 100 safety factor = 10 mcg/kg/day
(600 mcg/day/60 kg person)

The portion of the ADI set aside for milk is 4%. Consequently, the ADI for cattle is allocated in the following manner:

ADI (milk) = 0.4 mcg/kg/day (24 mcg/day/60 kg person)
ADI (tissues) = 9.6 mcg/kg/day (576 mcg/day/60 kg person)

Safe Concentrations (SC's) for Cattle Tissues and Milk:

SC (milk) = 0.4 mcg/kg x 60 kg/1.5L
= 16 mcg/L or 16 ppb

SC (muscle) = 9.6 mcg/kg x 60 kg/0.3 kg
= 1920 mcg/kg = 1920 ppb or 1.92 ppm

SC (liver) = 9.6 mcg/kg x 60 kg/0.1 kg
= 5760 mcg/kg = 5760 ppb or 5.76 ppm

ATTACHMENT D

INAD No. 8058

DATE	OCCURRENCE
6/22/90	Merck initiated multigeneration study in rats (preclinical safety study) (completed 6/20/91)
7/9/90	Merck initiated preclinical safety study (completed 8/3/90)
10/2/90	Merck initiated preclinical safety study (completed 11/2/90)
2/25/91	Merck submitted residue chemistry protocol to establish INAD.
2/28/91	FDA assigned INAD No. 8058.
5/11/91	FDA responded to 2/25/91 submission of residue chemistry protocol.
6/11/91	Merck submitted first phase of original NADA including results of preclinical safety studies.
6/20/91	Merck completed multigeneration study in rats.
7/8/91	Merck submitted residue chemistry protocol.
8/20/91	FDA requested methods used in residue chemistry protocol.
11/8/91	FDA responded to submission of first phase of original NADA
1/2/92	FDA further responded to submission of first phase of original NADA.
1/31/92	Merck responded to FDA letter regarding preclinical safety study
5/5/92	Merck submitted four protocols for environmental studies.
5/6/92	Merck submitted protocol for residue chemistry study
6/26/92	Merck responded to FDA letter regarding preclinical safety studies
8/17/92	Merck submitted draft protocol for target animal safety study
8/18/92	Merck submitted draft protocls for target animal safety study
8/19/92	Merck submitted protocol for target animal safety study
8/25/92	Merck submitted residue chemistry study protocol
12/7/92	Merck submitted Notice of Claimed Investigational Exemption of New Animal Drug.
12/15/92	Merck submitted efficacy protocol
12/16/92	Merck submitted efficacy protocol
12/17/92	Merck submitted efficacy protocols
12/18/92	Merck submitted efficacy protocol
12/21/92	Merck submitted efficacy protocol
12/21/92	Merck submitted results of preclinical safety study.
12/22/92	Merck submitted clinical efficacy protocol.
1/29/93	Merck responded to FDA letters regarding preclinical safety study
3/15/93	FDA responded to submission of Notice of Claimed Investigational Exemption.
6/16/93	Merck submitted residue chemistry study protocol.
7/28/93	Merck submitted phased data submission of preclinical safety study.
8/13/93	Merck submitted preclinical safety study protocol
8/30/93	Merck submitted amended slaughter authorization request.
9/1/93	Merck submitted environmental safety data

INAD No. 8058 (con't)

9/2/93	Merck submitted environmental safety study
9/3/93	Merck submitted environmental safety study
9/19/93	Merck submitted revised efficacy protocol
9/20/93	Merck submitted revised efficacy protocol
9/21/93	Merck submitted revised efficacy protocol
9/22/93	Merck submitted revised efficacy protocol
9/23/93	Merck submitted revised efficacy protocol
9/24/93	Merck submitted revised efficacy protocol
9/25/93	Merck submitted revised efficacy protocol
9/26/93	Merck submitted revised efficacy protocol
9/27/93	Merck submitted revised efficacy protocol
9/28/93	Merck submitted efficacy protocol.
10/6/93	Merck submitted request for expedited review status.
10/19/93	Merck submitted revised protocol for residue chemistry
10/20/93	Merck submitted residue chemistry protocol.
10/22/93	Merck submitted manufacturing chemistry study protocol.
11/16/93	Merck submitted environmental safety information
1/5/94	FDA granted expedited review status.
1/14/94	Merck submitted residue chemistry protocol
2/22/94	Merck submitted residue chemistry protocol
2/28/94	Merck responded to FDA letter on preclinical safety study review
3/17/94	Merck submitted amended slaughter authorization request
3/18/94	Merck submitted residue chemistry report.
3/25/94	FDA authorized investigational withdrawal periods
4/18/94	Merck submitted residue chemistry study protocol
4/21/94	FDA issued revised authorization
4/22/94	Merck submitted residue chemistry information
4/22/94	Merck submitted authorization request
4/25/94	Merck submitted efficacy study protocol meeting minutes
5/2/94	Merck submitted target animal safety study report
5/3/94	Merck submitted protocol for target animal safety study.
5/4/94	Merck submitted protocols for environmental studies
5/17/94	Merck submitted efficacy data
6/17/94	Merck submitted efficacy data
6/29/94	Merck submitted amendment to target animal safety
7/6/94	Merck submitted residue chemistry study
7/11/94	Merck responded to FDA letter regarding preclinical safety study
7/12/94	Merck submitted chemistry information
7/13/94	Merck submitted environmental study report
7/13/94	Merck submitted preclinical safety study report
7/26/94	FDA responded to amended authorization
8/3/94	Merck submitted environmental safety studies.

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8/11/94	Merck submitted preclinical safety study
8/11/94	Merck submitted environmental safety study
8/12/94	Merck responed to FDA letter concerning preclinical safety study
8/19/94	Merck submitted environmental safety information
8/23/94	Merck submitted amended authorization request
9/15/94	Merck responded to FDA letters concerning residue chemistry information
10/27/94	Merck submitted preclinical safety study
11/9/94	Merck responded to FDA letter concerning preclinical safety study
11/10/94	Merck submitted safety information
12/6/94	Merck submitted report for residue chemistry study
12/8/94	Merck responded to amended FDA authorization letter
2/3/95	Merck responded to FDA letter regarding residue chemistry information
2/23/95	Merck submitted environmental safety study
2/24/95	Merck responded to FDA letter concerning residue chemistry information
2/24/95	Merck submitted environmental safety study
2/27/95	Merck submitted residue chemistry study
2/27/95	Merck submitted environmental safety study
2/28/95	Merck submitted residue chemistry study
2/28/95	Merck responded to FDA letter regarding preclinical safety study
3/8/95	FDA responded to amended FDA authorization letter
3/17/95	Merck submitted residue chemistry study
3/17/95	Merck submitted environmental safety study
3/20/95	Merck submitted residue chemistry information
03/21/95	Merck requested to discuss preclinical safety study
3/21/95	Merck requested meeting to discuss residue chemistry
4/5/95	Merck submitted residue chemistry study
4/12/95	Merck submitted target animal safety study
4/13/95	Merck submitted target animal safety study
4/18/95	Teleconference regarding meeting request
4/24/95	Merck submitted amendment to residue chemistry information
4/24/95	Merck submitted amended authorization request
5/10/95	FDA sent minutes of residue chemistry meeting
06/6/95	Merck responded to FDA letter regarding efficacy studies
6/6/95	Merck submitted target animal safety information
6/6/95	Merck submitted amendment to target animal safety study
6/29/95	Merck submitted residue chemistry study
6/29/95	Merck submitted environmental safety study
6/30/95	Merck submitted environmental safety study
07/5/95	Merck responded to FDA letter concerning environmental safety study

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7/13/95	FDA confirmed human food safety requirements
7/20/95	FDA agreed with proposal to amend authorization
7/25/95	Merck submitted residue chemistry protocol
7/28/95	Merck submitted residue chemistry protocol
7/31/95	Merck submitted efficacy study
8/2/95	Merck submitted efficacy study
8/14/95	Merck submitted efficacy study
8/17/95	Merck submitted efficacy study
8/18/95	Merck submitted efficacy study
8/24/95	Merck submitted residue chemistry protocol
8/28/95	Merck submitted meeting request
9/13/95	Merck submitted residue chemistry information
9/15/95	Merck responded to FDA letter regarding target animal safety study
9/15/95	Merck submitted amendment to efficacy study
9/15/95	FDA provided comments on residue chemistry protocol
09/18/95	Merck submitted environmental safety information
9/29/95	Merck submitted manufacturing, facilities and controls information
10/10/95	Merck submited efficacy trials
10/11/95	FDA provided CVM review of residue chemistry study
10/12/95	Merck submitted residue chemistry information
11/16/95	Merck submitted residue chemistry study
11/16/95	Merck responded to FDA letter concerning residue chemistry information
11/16/95	Merck submitted efficacy study
11/17/95	Merck submitted residue chemistry study
12/7/95	Merck provided additional residue chemistry information
12/21/95	Merck submitted residue chemistry study
12/27/95	Merck submitted residue chemistry in responded to FDA letter
1/4/96	Merck submitted target animal safety study
1/17/96	Meeting with FDA on efficacy data
2/14/95	FDA provided minutes of teleconference on residue chemistry study
2/15/96	Merck confirmed meeting to discuss preclinical safety study
2/15/96	Merck responded to FDA letter regarding manufacturing chemistry
2/16/96	Merck responded to FDA letter regarding efficacy trials
2/20/96	FDA requested additional information regarding a target animal safety study FDA responded to residue chemistry information
3/25/96	Merck submitted efficacy study protocol
3/29/96	Merck submitted meeting request to discuss target animal safety study
4/1/96	Merck submitted meeting request for residue chemistry
4/3/96	Merck submitted efficacy study
4/8/96	Merck submitted minutes preclinical safety study meeting

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4/12/96	Merck submitted response to FDA letter regarding efficacy trials
4/15/96	Merck submitted minutes of teleconference regarding manufacturing chemistry
4/17/96	Merck submitted environmental safety study report
4/23/96	Merck submitted residue chemistry protocol
5/6/96	Merck submitted environmental safety study report
5/7/96	Merck submitted residue chemistry information
5/7/96	Merck submitted environmental safety information
5/7/96	FDA provided minutes of teleconference on manufacturing chemistyr
5/10/96	Merck responded to FDA letter regarding clinical efficacy trials
5/13/96	Merck responded to FDA letter regarding manufacturing chemistry
5/23/96	FDA sent technical section complete letter
5/28/96	Merck responded to FDA letter regarding efficacy trial
5/29/95	FDA provided minutes of residue chemistry meeting
6/21/96	Merck responded to FDA letter concerning efficacy trial
6/24/96	Merck responded to FDA letter regarding manufacturing chemistry
6/26/96	Merck submitted meeting minutes concerning residue chemistry
6/27/96	Merck submitted amendment to environmental safety information
6/28/96	Merck submitted efficacy study
7/3/96	Merck submitted efficacy study
7/8/96	Merck submitted residue chemistry study
8/6/96	Merck responded to FDA letter concerning efficacy trial
8/16/96	Merck submitted efficacy study
8/28/96	Merck submitted residue chemistry information
9/4/96	Merck submitted efficacy study
9/11/96	Merck submitted labeling
9/12/96	Merck submitted target animal safety information
9/13/96	Merck submitted residue chemistry information
9/20/96	Merck submitted draft FOI summary
9/26/96	Merck responded to FDA letter concerning residue chemistry
9/26/96	Merck submitted manufacturing chemistry data
10/11/96	Merck responded to FDA letter regarding efficacy data
10/29/96	Merck submitted residue chemistry study
10/29/96	Merck submitted preclinical safety study
10/29/96	Merck submitted residue chemistry data
11/7/96	Merck submitted efficacy trials
11/11/96	Merck submitted environmental safety information
11/11/96	Merck submitted patent No. 4,427,663 expiration date March 16, 2002
11/21/96	Merck submitted residue chemistry data
12/18/96	Merck submitted manufacturing chemistry information
12/19/96	Merck submitted residue chemistry information

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1/20/97	Merck submitted residue chemistry information
1/31/97	Merck responded to FDA letter on labeling
2/7/97	Merck responded to FDA letter on labeling
2/7/97	Merck submitted amendment to patent information submission
2/10/97	Merck submitted amendment to a submission of final labeling.
2/10/97	FDA indicated Human Food Safety Technical Section Complete FDA accepted residue chemistry studies
2/12/97	Merck submitted final labeling
3/5/97	FDA responded to submission on FOI summary FDA confirmed labeling and FOI sections of NADA complete
3/6/97	FDA confirm manufacturing chemistry section of NADA complete

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DATE	OCCURENCE
3/27/97	Merck submitted original New Animal Drug Application
4/16/97	FDA approved NADA